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FORMULATION AND DEVELOPMENT OF SELF EMULSIFYING DRUG DELIVERY SYSTEM OF RITONAVIR.

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ABSTRACT

SE system is isotropic blend of drug, oil, surfactants and/or co solvents forming O/W emulsion in GI fluids, with mild agitation by GIT. Formed o/w emulsions have more stability in thermodynamic way as small amount of dispersed oil, fine sizes of droplets; more interface area improves activity & decreases drug-gut wall irritation. Therefore, SEDDS is competent system for BCS class II & IV drugs. Pre formulation studies like appearance, melting point, pH and spectroscopy of ritonavir showed that sample was pure. Calibration curve of Ritonavir was generated in different mediums, which showed that drug followed beer lamberts law. Saturated solubility study of Ritonavir in different vehicles showed that drug posses' high solubility in oleic acid, Tween 80 and Capmul MCM. From pseudo ternary phase diagram, highest micro emulsion zone found was at Smix ratio (1:3). From pseudo ternary diagram, quantitative selection of vehicles was done and liquid SMEDDS formulations of Ritonavir were prepared (S1to S5) by varying concentration of oil and Smix. The optimized SMEDDS S2 contained Oleic acid (28.8 %W/W), TWEEN 80 (16.8 %W/W) and CAPMUL MCM (50.4 % W/W). The SMEDDS S2 showed high negative zeta potential near to range, homogenous globule distribution (from PDI), smaller droplet size, high % T and % drug content in comparison to S1 and S3. S2 showed drug release of 99.77 % whereas pure drug showed 34.99 % at 30 min in Ph 1.2. S2 showed drug release of 99.76 % whereas pure drug showed 56.18 % at 30 min in pH 7.0 buffer with SLS, S2 showed drug release of 99.7 % whereas pure drug showed 35.02% at 30 min in pH 7.0 buffer without SLS, which indicate drastic improvement in drug release from liquid SMEDDS S2 in comparison to pure drug and even pure drug showed effect of SLS in pH 7.0 buffer on release of drug, while liquid SMEDDS didn't showed effect of pH and SLS on release of drug. S2 showed high % drug release in comparison to S1 and S3.

Keywords: Acne vulgaris, Moisturizer, Spreadability, Viscosity.

INTRODUCTION

SEDDS are isotropic type of mixtures composed of drug, one or more lipids (natural/ synthetic oils), hydrophilic/lipophilic surfactants, co-surfactants and/or co solvents, with some gentle agitation and GI motility followed by aqueous medium dilution of GIT, systems instantaneously form very fine (o/w) emulsion or micro emulsion. Droplets passes through the stomach and drug distributed through GIT minimizing the drug contact to gut wall and thereby irritation due to prolonged contact of them. The broad term 'SEDDS' produces o/w emulsions having few µm to nm droplet size. SMEDDS are the formulations, which forms micro emulsions having 100-250 nm droplets size & transparent. SNEDDS is the recent approach having droplet size <100 nm. SMEDDS are metastable, sensitive but physically stable dispersion formulation, their manufacture is easy [Kohli et al., 2010].

SEDDS system offer a small globule size, huge interfacial area to provide oil and water partitioning of drug compared to oily solutions. The system increases pancreatic lipase activity for hydrolyzation of triglycerides, formation of bile salts micelles having drug and promoting the faster drug release. In addition to it, surfactant in the formulations improves the oral drug bioavailability by mechanisms like maintainance of drug in dissolved form without dissolution step and by enhancing intestinal permeability. Oil in formulation uniform and rapid drug distribution in the GIT, and minimizes the contact and drug irritation to gut wall. Even, lipids protect the drug from the enzymatic and/or chemical degradation activates lipoproteins and improves

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drug lymphatic transport and increases its oral bioavailability. Then liquid SEDDS formulation can be incorporated into HGC/SGC or may be converted to solids like powder, granules or pellets to fill in capsules or can be made tablets by the methods like melt granulation or extrusion, spray drying or cooling, adsorption on solid & supercritical fluid methods [Vemula, 2010].

By considering some above-mentioned needs of study, the objectives and the plan of present work is to develop liquid SMEDDS of Ritonavir and to characterize liquid SMEDDS for their globule size and zeta potential determination, polydispersibility index, viscosity and refractive index, % Transmittance, drug content and thermodynamic stability study and in vitro release and optimization of liquid SMEDDS for ritonavir.

MATERIALS AND METHODS

Calibration curve of Ritonavir in 1.2pH Hydrochloric acid buffer (0.1N HCl)

10mg of drug was transferred into 100ml volumetric flask and then made upto 100ml with 10ml methanol and 90ml 0.1N HCl. From this 2, 4, 6, 8 and 10ml was taken and made upto 10ml with 0.1N HCl and their absorbance was measured at 246nm [Julianto et al., 2000].

IR Spectroscopy

IR spectroscopy is a key analytical method for chemical identification. The drug polymer interaction can also be studied by FTIR spectroscopy. Fourier–transform infrared (FT–IR) spectrum of moisture free powdered samples of pure Ritonavir was recorded by FTIR spectrophotometer by KBr pellet method. Scan range set at 400–4000 cm^{-1} and the resolution set at 1 cm^{-1} and the spectrum analysis was done for identification of sample.

Determination of the saturation solubility of Ritonavir in Vehicles and screening of vehicles

Ritonavir solubility in various vehicles like oils, surfactants, and co surfactants was checked, respectively. Excess drug amount was added in 2 ml of each individual vehicle contained in stoppered vial separately and after sealing, which was heated (40ºC) & sonicated for solubilization. Vials were then shaken at 37°C±1°C and then allowed them for equilibrium. Then samples were centrifuged (5000 rpm) for 5-10 min to separate the undissolved drug and the supernatants were filtered by membrane filter (0.45µm, 13mm, Whatman, India) and after appropriate dilution with methanol, the absorbance was measured against respective blank by UV spectroscopy at λ max. The concentration of ritonavir was calculated by the using calibration curve. Solubility was checked for three times and SD was calculated.

Screening of surfactant for emulsifying ability with oil Surfactant was mixed with oil in ratio of 2:1.Mixture was homogenized with gentle heat 25-35 ºC. The mixture, 50mg, weighed precisely and diluted upto 50ml to get fine emulsion. Even no. of inversions was done for each surfactant to get uniform emulsion. After 2 hrs of equilibrium, % T was also measured.

CONSTRUCTION OF PSEUDO TERNARY PHASE DIAGRAMS

For optimization of excipient concentration, different batches of varied concentration were prepared & titrated with distilled water until turbidity disappeared. Two dimensional ternary phase diagram were prepared by using a constant ratio of surfactant: co-surfactant. Based on solubility observations, excipients having highest drug solubility were selected for self-emulsifying system. The PDs of oil, Smix & water were drawn using water titration technique. For PD at specific Smix ratio 1:1, 1:2, 1:3, 1:4, 2:1, 3:1, 4:1(w/w) were mixed together, which were blended with oil in a proportions of oil: Smix as 9:1, 8:2, 7:1, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9 with constant stirring by magnetic stirring and then The mixture blend of oil and surfactant: co-surfactant was titrated with distilled water in increment of 0.5% (W/W) with proper mixing. Each system was allowed to have equilibrium and then checked for phase clarity, transparency & flow ability. Water conc. at which gel formation, turbidity-transparency transition done was noted, which was being used to check the boundaries of micro emulsion diagram with reference to chosen value of oils and Smix ratio. For each system for all weight ratios of oil, surfactant, co-surfactant, point indicating clear isotropic mixtures were selected and PDs were constructed by chemix software, in which clear micro emulsion region in PDs were being observed and concentration of excipients can be optimized quantitatively for formulation development of SMEDDS.

Pseudo ternary phase diagram study of Ritonavir

Based on the data observations of solubility studies of Ritonavir, Oleic acid (oil), Tween 80 (Surfactant) and Capmul MCM (Co-surfactant) were selected as for phase diagram study. Here seven ratios of surfactant: co-surfactant (Smix) (1:1, 1:2, 1:3, 1:4, 2:1, 3:1, 4:1 W/W) were mixed together. Each ratio of Smix blended with Oleic acid in a proportion of 9:1, 8:2, 7:1, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9 with constant stirring using magnetic stirrer. Blend was titrated by distilled water in increment of 0.5% (W/W) with proper stirring to form clear transparent microemulsion. The point which indicates the clear and isotropic mixtures were considered to be within the microemulsion region. Optimization of the concentration of vehicles (Oleic acid, Tween 80, Capmul MCM) was based on maximum uptake of water by formed micro emulsion. Drug loading capacity was checked on self-micro emulsifying domain of ternary phase diagram.Effect of Drug for selected PD to get drug loading capacity

This is to investigate the effects of Ritonavir on the selected respective phase diagrams. Drug dose was added to boundary contents of microemulsion domain of

PDs. SE performance was checked after water dilution. At first 10 mg drug was added in plain SMEDDS formulation & allowed it to dissolve in. SMEDDS was diluted using purified water & keep it over night at room temperature. After that, visual inspection was carried out. If dilution was transparent, more amount of drug in increment of 10 mg was added. This procedure was repeated up to dilution was turbid & found out maximum drug loading capacity of plain selected SMEDDS.

Formulation of liquid SMEDDS of Ritonavir

Based on the area of high microemulsion region from the phase diagrams, Smix ratio of 1:3 were selected for the formulation development of ritonavir SMEDDS. From this PD, % water, oil and Smix from each selected ratios were determined and ritonavir SMEDDS formulations S1, S2, S3, S4 and S5 were selected for formulation and for further evaluation studies. Here, ritonavir SMEDDS were developed by Oleic acid, Tween 80 and Capmul MCM as respective oil, surfactant and co-surfactant with 1:3 Smix ratio with the oil range 20-40% and Smix range 60-80%. The weight of the formulation was kept approximately 580 mg for unit dose. Amount of ritonavir in all the formulation was kept constant (100 mg). Ritonavir was weighed accurately and mixed with required quantity of oleic acid (oil) in a glass vial with gentle stirring and vortex mixing. Respective required quantity of Tween 80 (surfactant) and Capmul MCM (co-surfactant) added to the vial and mixed by vortex mixing till clarity. The blend was stored at RT. SMEDDS were checked for turbidity/phase separation before evaluation studies like self-emulsification, % T, % drug content, particle size and in vitro release study.

EVALUATION/CHARACTERIZATION OF LIQUID SMEDDS [Parmar et al., 2012]

Macroscopic evaluation by visual assessment and robustness to dilution with pH effect

SMEDDS (approximately 0.2ml) was diluted with distilled water (100 ml) and gently stirred with glass rod or by magnetic stirrer at 100 rpm. Temperature should be 37°C. Macroscopic assessment like self-emulsification efficiency, appearance in terms of color, transparency, phase separation and precipitation of API was carried out visually immediately after dilution. Color, transparency or phase separation change in microemulsion during normal storage (37±2°C) was observed. All SMEDDS were diluted by pH 7.0 phosphate buffer for ritonavir and microemulsions were checked for precipitation or phase separation after 24 hours. Precipitation was checked after 24 hr to categorize the formulation clear (transparent and blue tint), nonclear or turbid; stable (if no precipitation after 24 hr); unstable (if precipitates within 24 h).

Dispersibility test and Determination of self-emulsification time

Emulsification time was checked in USP dissolution apparatus. 300mg SMEDDS was added to 500ml water gradually at 37 °C. Dissolution paddle rotation provided gentle agitation (50 rpm). Time for emulsification observed visually and appearance compared for grading system [Parmar et al., 2015].

%Transmittance test [Raval et al., 2012]

Stability of optimized SMEDDS microemulsion with dilutions was observed by measuring %T. 1 ml SMEDDS diluted to 100 ml with distilled water & %T measured at 246 nm in UV spectrophotometer and for each sample triplicates were performed.

Droplet/Globule size measurement

Globule Size analysis of micro emulsion of SMEDDS carried out by dynamic light scattering with Malvern Zetasizer. SMEDDS taken in 100 ml distilled water, at 37 ± 0.5˚C and emulsion made by magnetic stirrer agitation. Samples were placed in square glass cuvettes & droplet size analysis was carried out.

Zeta potential measurement

Zeta potential for micro emulsion was determined using Malvern Zetasizer. SMEDDS were dispersed in distilled water, at 37 ± 0.5˚C and emulsions were prepared by gentle agitation using a magnetic stirrer. Samples put in methanol & sample rinsed zeta cells & results recorded.

Refractive index measurement

RI of SMEDDS with drug and without drug was measured using Abbes refractometer.

Viscosity determination [Julianto et al. 2000]

Rheological properties of microemulsion were being evaluated. SMEDDS (0.5 g) 10 times diluted with distilled water with magnetic stirring & SMEDDS and microemulsion viscosity was being determined using Brookfield viscometer (DVIII+ Rheometer) at room temperature.

In-vitro dissolution study [Julianto et al. 2000]

In vitro dissolution study for Ritonavir formulations (S1, S2, S3 and marketed tablet and pure drug). In vitro dissolution was done in 900ml medium (pH 1.2, pH 7.0 phosphate buffer with and without 0.5% SLS) at 37 ± 0.5°C by USP method (dissolution type I, at 100 rpm). Ritonavir SMEDDS (S1, S2, S3) filled in HGC was put in basket during release period one by one and release profile were compared with conventional marketed tablet. 10ml sample was withdrawn at 0, 5, 10, 15, 20, 30, 45 min & filtered through a 0.45 µm filter, diluted & analyzed spectrophotometrically at λ max using calibration curve. Same amount dissolution medium replaced at a time after test withdrawal. This is done to maintain the volume. In vitro dissolution was carried out three times & mean

value was tabulated. % drug dissolved at time intervals tabulated by respective calibration curve.

RESULTS AND DISCUSSION

Characterization of Ritonavir

The characterization of pure Ritonavir was carried by performing tests like organoleptic properties, melting point determination, solubility analysis, pH, loss on drying, etc.

Results of characterization tests (especially loss on drying and melting point) of Ritonavir pure drug complied with the specifications of pharmacopoeia or other reliable material and with certificate of analysis given by sample provider of Ritonavir.

Spectroscopic Study of Ritonavir

The calibration data of Ritonavir was subjected to liner regression. The calibration range was found to be 20 to 100 µg/ml with R^2 value of 0.9998. Slope of the regression was found to be 0.0124 with intercept of regression line was found to be 0.0008.

From the ritonavir FTIR spectrum compared with standard, It was found that sample of ritonavir was identified as a pure compound.

Determination of the saturation solubility of Simvastatin in Vehicles and screening of vehicles

Various oil, surfactant & co-surfactant were screened by solubility of Simvastatin in different vehicles as per the solubility method described, solubility of Simvastatin in different vehicles were determined using UV method and using calibration curve in methanol.

Solubility of Ritonavir in different co-surfactants

As per solubility data of Ritonavir in different oils, maximum amount of Ritonavir dissolves in Oleic acid. So Oleic acid was selected as oil having Ritonavir solubility of 73.667±1.5 mg/ml for ternary phase diagram. As per solubility data of Ritonavir in different surfactants, maximum amount of Ritonavir dissolved in Tween 80. So Tween 80 was screened as surfactant having Ritonavir solubility of 85±1.7 mg/ml for ternary phase diagram. As per solubility data of Ritonavir in different co-surfactants, maximum amount of Ritonavir dissolved in Capmul MCM. So Capmul MCM was selected as co-surfactant having Ritonavir solubility of 103.333±1.2 mg/ml for ternary phase diagram. Emulsifying ability with Oleic acid: The % transmittance values and number of inversions required for uniform emulsions given in Table.

Tween 80 has good ability to emulsify Oleic acid; even number of inversions required for formation of uniform emulsion with Tween 80 was less with high % transmittance. So Tween 80 as surfactant was confirmed.

CONSTRUCTION OF TERNARY PHASE DIAGRAMS FOR DIFFERENT SYSTEMS

For optimization of excipient concentration for Ritonavir SMEDDS, ternary diagrams generated by water titration technique using chemix or Sigma plot 11.0 software & from it, highest micro-emulsion zone (region) were found out to select optimum excipient concentration.

From the Ritonavir ternary phase diagrams shown, the highest micro emulsion zone was found in S mix =s/c = 1:3 (phase diagram no.2 from table) in comparison to other phase diagrams. So phase diagram 2 was selected for preparation of Ritonavir liquid SMEDDS.

From drug on phase diagram study, it was concluded that transparent micro emulsion was obtained while maximum 200 mg Ritonavir loading. So maximum up to 200 mg drug loading is possible in these SMEDDS systems.

FORMULATION OF LIQUID SMEDDS

Based on the area of high micro emulsion region from the phase diagrams, Ritonavir SMEDDS formulations S1, S2, S3, S4 and S5 were successfully prepared without turbidity or phase separation using oil Oleic acid, surfactant Tween 80 and co-surfactant Capmul MCM with Smix ratio of 1:3. The weight of the formulation was kept approximately 580 mg. Amount of Ritonavir in all the formulation was kept constant (100 mg).

EVALUATION/CHARACTERIZATION OF LIQUID SMEDDS

The evaluation tests were performed for selected formulations of SMEDDS of Ritonavir (S1, S2, S3, S4 and S5).

Macroscopic evaluation by visual assessment

Macroscopic assessment like self-emulsification efficiency, appearance in terms of color, transparency, phase separation and precipitation of API was carried out visually immediately after dilution and after 24 hrs for all selected SMEDDS even after dilutions.

Formulations S4, S5 were turbid and unstable emulsions and even showed phase separation and precipitations. So they were not taken for dilution and pH effect study. Formulation S3 were less clear and unstable emulsion. Formulations S1, S2 were clear, good & stable without any sign of precipitation even after 24 hrs of dilution.

SMEDDS S4, S5 were affected by dilution with any mediums (distilled water or pH 1.2 or pH 7.0 medium). S1, S2, S3 Ritonavir were robust to dilution with all mediums without precipitation and phase separation. Even there was no significant effect of pH found on any SMEDDS, because non-ionic surfactants have very very less effects of pH.

Dispersibility test and Determination of self-emulsification time

Emulsification time is most important parameter for SMEDDS and micro emulsion formulation. From Dispersibility test and self-emulsification time, it was

found that Formulation S5 forms greyish white emulsions with oil droplets (Grade IV) having > 3min self-emulsification time. Formulation S4 forms milky white grade III emulsion with self-emulsification time near to 3 min. We are rejecting the formulations S5, for next studies like % transmittance test and Globule size. Formulation S3 forms bluish white (Grade III) emulsion with emulsification time of 2 min. Formulations S1 forms slightly bluish (Grade II) micro emulsion with emulsification time of 1-2 min. Formulations S2 forms (Grade I) micro emulsion spontaneously within 1 min. Less than 1 minute self-emulsification time is required to form good SMEDDS. So Formulation S2 for ritonavir SMEDDS, was further studied for next characterization studies.

%Transmittance test

From the above table, it was seen that formulations S4, S5 were milky white and unclear showing very less % Transmittance values which are unacceptable and hence they were not undergone to further characterization studies. Formulation S3 shows very less % transmittance, so they are somewhat unclear. Formulations S1 shows % transmittance lesser. Formulations S2, shows % Transmittance near to 100% so they are very clear & transparent & does not affected when diluted with SGF.

Droplet/Globule size measurement

From the table and images, it was seen that formulations S1,S3 shows large particle size comparative to S2, Formulation, means that globule sizes increases when oil conc. increases & surfactant conc. decreases. Droplet size is smaller, interfacial area is larger for drug absorption. Here, SMEDDS formulations S2, showed very small droplet size and even upon 100 ml dilution with water globule sizes did not changes. This suggests that upon dilution with gastric fluid in body, optimized micro emulsion formulation will remain stable & will not convert into macro emulsion.

Polydispersity index (PI) is particle homogeneity measurement varies from 0-1. Higher the value of PI, lower the uniformity of particles/globules in formulation. Closer PI to zero, particles are much homogenous. Here for all SMEDDS except formulation S3 PI values are less than 1 and also near to zero; formulation S3 showed PI 0.418, which were not in acceptable limits. So in all formulations except S3, particles or globules were homogenous and uniformly distributed throughout the formulations.

Zeta potential measurement

Zeta potential of all three in 100 times water diluted samples of SMEDDS formulations are shown in table. Stability of colloidal system is dependent on magnitude of zeta potential. If particles have high negative or high positive zeta potential, it will give more stability to dispersion due to repelling of particles/globules and if particles have less zeta potential, then less force is available to repel particles/globules so particles comes together and leads to instability of dispersion. +30 or −30mV zeta potential value is dividing line for deciding stable or unstable dispersion. Here, from the table, formulations S2, have high magnitude of negative zeta potential which is near to range.

Table 1: Formulations of Ritonavir liquid SMEDDS

Formulation Batches of SMEDDS of Simvastatin (w/w)					
	S1	**S2**	**S3**	**S4**	**S5**
OIL %+ SMIX %	25+75	30+70	35+65	20+80	40+60
Drug	100	100	100	100	100
Oil	120	144	168	96	192
S mix (1:3)	360	336	312	384	288
Surfactant	90	84	78	96	72
Co surfactant	270	252	234	288	216
Total	580	580	580	580	580

Unit dose formulation in single hard gelatin capsule = 580 mg; O = oil (Oleic acid) , S = Surfactant (Tween 80) , C = Co-surfactant (Capmul MCM)

Table 2: Characterization of Ritonavir

Characters	**Criteria**	**Result**
Appearance	White to off white powder	White to off white powder
Taste	Bitter	Bitter
Melting range	126-132 ^{o}C	126-132 ^{o}C
Solubility	Water solubility sparing Methylene insoluble, slight in solubility in chloride. methanol,	Water insoluble (0.024 mg/ml), insoluble in HCl, soluble in methanol, ethanol (>25 mg/ml),

	Dissolves in 1M NaOH	chloroform,methylene chloride and PEG. It dissolves in 1M NaOH
pH of solution	-	4.5
Loss on drying	Should not be > 0.5 %	0.38%

Table 3: Saturation Solubility of Ritonavir in vehicles

Vehicle		**Solubility (mg/ml)**			**Average solubility(mg/ml)**	***Average solubility mean ± S.D (mg/ml)**
		I	**II**	**III**		
OIL	Olive oil	35	37	38	36.7	36.667±1.5
	Soyabean oil	25	27	26	26.0	26±1.0
	Sunflower oil	24	23	25	24.0	24±1.0
	Peanut oil	18	18	20	18.7	18.667±1.2
	Cottonseed oil	30	30	31	30.3	30.333±0.6
	Sesame oil	33	35	34	34.0	34±1.0
	Oleic acid	75	72	74	73.7	73.667±1.5
	Captex 100	17	19	16	17.3	17.333±1.5
	Captex 200	22	25	23	23.3	23.333±1.5
	Captex 355	36	38	35	36.3	36.333±1.5
SURFACTANT	**Tween 80**	86	86	83	85.0	85±1.7
	Cremophore ELP	39	42	40	40.3	40.333±1.5
	Cremophore RH 40	70	72	72	71.3	71.3±1.2
	Labrafil M2125CS	19	19	15	17.7	17.667±2.3
	Labrasol	55	55	53	54.3	54.333±1.2
	Span 20	30	30	28	29.3	29.333±1.2
	Tween 20	57	56	59	57.3	57.333±1.5
	Span 80	23	24	22	23.0	23±1.0
	Acrysol K-140	34	33	34	33.7	33.667±0.6
	Labrafac CC	24	23	22	23.0	23±1.0
CO-SURFACTANT	Propylene glycol	40	40	36	38.7	38.667±2.3
	PEG 400	69	69	70	69.3	69.333±0.6
	Capmul MCM	104	104	102	103.3	103.333±1.2
	Lauroglycol 90	53	52	53	52.7	52.667±0.6
	Capryol 340	28	27	26	27.0	27±1.0
	Peceol	49	48	46	47.7	47.667±1.5
	Acconon MC 82	61	62	60	61.0	61±1.0
Oil	**Oleic acid**	**75**	**72**	**74**	**73.7**	**73.667±1.5**
surfactant	**Tween-80**	**86**	**86**	**83**	**85.0**	**85±1.7**
Co-surfactant	**Capmul MCM**	**104**	**104**	**102**	**103.3**	**103.333±1.2**

Table 4: Emulsification efficacy of surfactant with Oleic acid

Surfactant	**% Transmittance**	**No. of inversions**
Tween 80	99.1	5

Table 5: List of phase diagrams for different ratios of Smix and for Ritonavir SMEDDS

PD No.	**Drug**	**Oil**	**Surfactant**	**Co-surfactant**	**Smix**
1	Rito	Oleic acid	Tween 80	Capmul MCM	1:4
2	Rito	**Oleic acid**	**Tween 80**	**Capmul MCM**	**1:3**
3	Rito	Oleic acid	Tween 80	Capmul MCM	1:2
4	Rito	Oleic acid	Tween 80	Capmul MCM	1:1
5	Rito	Oleic acid	Tween 80	Capmul MCM	2:1
6	Rito	Oleic acid	Tween 80	Capmul MCM	3:1
7	Rito	Oleic acid	Tween 80	Capmul MCM	4:1

Table 6: Ternary phase diagrams for Ritonavir SMEDDS

Smix	**(1:1) (%)**			**(2:1) (%)**			**(3 :1) (%)**			**(4 :1) (%)**		
O+ Smix	**O**	**E**	**W**	**O**	**E**	**W**	**O**	**E**	**W**	**O**	**E**	**W**
5 +95	0.05	0.94	99.01	0.05	0.94	99.01	0.05	0.94	99.01	0.05	0.94	99.01
10 +90	0.1	0.89	99.01	0.1	0.9	99	0.1	6.9	93	0.1	9.9	90
15+85	0.15	0.84	99.01	3.25	3.74	93.01	4.49	9.48	86.03	5.73	17.11	77.16
20+80	4.33	5.28	90.39	5.54	6.79	87.67	6.98	11.89	81.13	11.22	15.32	73.46
25+75	5.76	7.91	86.33	7.93	10.67	81.4	13.49	12.18	74.33	15.17	14.28	70.55
30+70	7.94	11.88	80.18	9.48	12.38	78.14	14.94	21.99	63.07	18.59	25.99	55.42
40+60	17.77	27.6	54.63	19.85	28.73	51.42	22.99	30.12	46.89	29.64	35.93	34.43
50+50	37.5	27.9	34.6	36.55	34.97	28.48	38.84	40.54	20.62	42.21	39.89	17.9
60+40	47.22	32.27	20.51	48.9	33.2	17.9	52.95	33.88	13.17	54.87	32.44	12.69
70+30	52.15	28.77	19.08	60.36	27.23	12.41	65.11	24.34	10.55	68.18	21.89	9.93
80+20	71.73	19.38	8.89	72.62	18.18	9.2	76.14	15.09	8.77	79.92	11.61	8.47
90+10	81.81	9.09	9.1	82.6	9.09	8.31	82.97	9.38	7.65	83.65	9.79	6.56

Table 7: Ternary phase diagram for Ritonavir SMEDDS

Smix	**(1:2) (%)**			**(1:3) (%)**			**(1:4) (%)**		
O+ Smix	**O**	**E**	**W**	**O**	**E**	**W**	**O**	**E**	**W**
5 +95	0.05	0.94	99.01	**0.05**	**0.94**	**99.01**	0.05	0.94	99.01
10 +90	0.1	0.89	99.01	**0.1**	**0.89**	**99.01**	0.1	0.89	99.01
15+85	1.1	1.26	97.64	**0.15**	**0.89**	**98.96**	0.9	1.28	97.82
20+80	4.29	5.18	90.53	**0.16**	**0.92**	**98.92**	1.39	4.28	94.33
25+75	5.67	7.78	86.55	**0.24**	**0.88**	**98.88**	3.97	6.08	89.95
30+70	7.84	10.22	81.94	**0.3**	**0.7**	**99**	5.99	8.52	85.49
40+60	17.42	26.63	55.95	**4.67**	**6.22**	**89.11**	13.02	16.33	70.65
50+50	36.99	26.32	36.69	**6.7**	**6.44**	**86.86**	29.99	21.92	48.09
60+40	46.6	32.2	21.2	**23.4**	**19.11**	**57.49**	41.7	28.32	29.98
70+30	50.85	29.08	20.07	**47.66**	**20.09**	**32.25**	50.59	28.98	20.43
80+20	72.33	18.85	8.82	**62.44**	**17.54**	**20.02**	73.33	17.55	9.12
90+10	81.81	9.09	9.1	**81.82**	**9.09**	**9.09**	81.81	9.09	9.1

Table 8: Drug loading capacity of Ritonavir on selected phase diagram

Amount of Drug loaded (mg)	**Visual Inspection**
50	Transparent
100	Transparent
150	Transparent
200	Transparent
250	Turbid
300	Turbid
350	Turbid
400	Turbid
450	Turbid
500	Turbid

Table 9: Formulation Batches of liquid SMEDDS of Ritonavir

Formulation Batches of SMEDDS of Simvastatin (w/w)					
	S1	**S2**	**S3**	**S4**	**S5**
OIL %+ SMIX %	25+75	30+70	35+65	20+80	40+60
Drug	100	100	100	100	100
Oil	120	144	168	96	192
S mix (1:3)	360	336	312	384	288
Surfactant	90	84	78	96	72
Co surfactant	270	252	234	288	216
Total	580	580	580	580	580

O = oil (Oleic acid) , S = Surfactant (Tween 80) , C = Co-surfactant (Capmul MCM)
Unit dose formulation in single hard gelatin capsule = 500 mg

Table 10: Macroscopic evaluation of liquid Ritonavir SMEDDS

	S1	**S2**	S3	S4	S5
Colour	Uniform slight blue	Uniform	Uniform	Less uniform	Less uniform
Transparency	Uniform	Uniform	Less uniform	Less uniform	Less uniform
Phase separation	NO	NO	Very Slight	Clear separation	Clear separation
Precipitation	Clear, stable	Clear, stable	Clear ,stable	Turbid, Unstable	Turbid, unstable

Table 11: Dilution and pH effect on liquid SMEDDS

	Phase separation and precipitation						
	Dilution			Effect of pH			
	10 times	100 times	1000 times	water	pH 1.2	pH6.6 citrate buffer	pH7.0 buffer
S1	No	No	No	No	No	-	No
S2	No	No	No	No	No	-	No
S3	No	No	No	No	No	-	No
S4	No	Yes	Yes	Yes	Yes	-	Yes
S5	Yes	Yes	Yes	Yes	Yes	-	Yes

Table 12: Visual assessments of efficiency of self-micro emulsification of Ritonavir

Formulation	S1	**S2**	S3	S4	S5
Dispersibility	Rapid forming slightly bluish micro emulsion	Rapid forming micro emulsion	Bright white emulsion	White emulsion	Grey white emulsion with low emulsification time
Time of SE	within 1.1	Spontaneous within 30 seconds	2.1	2.9	> 3
Grade	II	I	III	III	IV

Table 13: %Transmittance of liquid SMEDDS of Ritonavir upon dilution with water and SGF

Formulation	***% Transmittance ± S.D**	
	Distilled water	**0.1 N HCl**
S1	88.82±0.08	88.86±0.015
S2	99.42±0.008	99.45±0.05
S3	77.76±0.098	78.10±0.004
S4	44.27±1.38	40.79± 2.365
S5	32.13 ± 1.16	35.89 ± 1.72

Table 14: Droplet size & Polydispersity index of SMEDDS formulations of Ritonavir

Formulation	**Particle size (nm)**	**Polydispersity index**
S1	19.07	0.273
S2	14.69	0.167
S3	41.7	0.418

Table 15: Zeta potential of liquid SMEDDS of Ritonavir

Formulation	**Zeta potential (mv)**
S1	-7.8
S2	-19.81
S3	NOT found

Table 16: Refractive index of liquid SMEDDS of Ritonavir

Formulation	***Refractive Index without drug**	***Refractive Index with drug**
S1	1.491 ± 0.07	1.495 ± 0.08
S2	1.385 ± 0.05	1.392 ± 0.04
S3	1.475 ±0.09	1.48 ±0.11

Table 17: Viscosity of liquid SMEDDS formulations of Ritonavir

Formulation	Viscosity (cps)	Viscosity of 10 times diluted (cps)
S1	218	21.40
S2	212	20.4
S3	225	23.32

Table 18: In-Vitro dissolution of Ritonavir SMEDDS (S1, S2 & S3), marketed tablet and Ritonavir drug in pH 1.2 medium

	Cummulative Percentage release (CPR)				
Time (min)	**S1**	**S2**	**S3**	**Tablet (100 mg)**	**Ritonavir Drug**
0	0	**0**	0	0	0
5	18.77±0.20	**22.99±0.35**	17.44±0.12	7.98±0.28	5.33±0.14
10	43.81±0.11	**50.72±0.29**	37.88±0.15	17.66±0.25	14.56±0.21
20	73.66±0.33	**84.7±0.2**	69.37±0.13	33.9±0.30	23.76±0.22
30	88.33±0.3	**99.77±0.22**	87.87±0.18	43.93±0.18	34.99±0.26
45	96.1±0.3	**99.92±0.14**	95.38±0.12	53.74±0.3	39.08±0.12

Table 19: In-Vitro dissolution of Ritonavir SMEDDS (S1, S2 and S3), marketed tablet and Ritonavir drug in pH 7.0 buffer with SLS

	Cummulative Percentage release (CPR)				
Time (min)	**S1**	**S2**	**S3**	**Tablet (100 mg)**	**Ritonavir Drug**
0	0	**0**	0	0	0
5	18±0.76	**23.72±1.01**	15.46±0.22	11.38±0.94	9.82±0.36
10	45.99±0.35	**51.92±0.63**	35.18±1.35	29.31±1.9	25.92±1.18
20	74.01±0.57	**86.29±0.35**	70.47±0.65	50.92±1.02	42.17±0.8
30	**87.21±0.42**	**99.76±0.21**	**88.26±0.39**	**70.37±0.45**	56.18±0.45
45	95.98±0.6	**99.92±0.33**	94.91±0.17	87.13±1.24	70.63±0.76

Table 20: In-Vitro dissolution of Ritonavir SMEDDS (S1, S2 & S3), marketed tablet and Ritonavir drug in pH 7.0 buffer without SLS medium

	Cummulative Percentage release (CPR)				
Time (min)	**S1**	**S2**	**S3**	**Tablet (100 mg)**	**Ritonavir drug**
0	0	**0**	0	0	0
5	18.02±0.43	**22.01±0.56**	16.91±0.37	8.82±0.39	6.12±0.28
10	45.81±0.33	**50.2±0.23**	39.88±0.17	22.93±0.26	15.87±0.29
20	74.86±0.36	**85.72±0.39**	70.37±0.6	39.44±0.32	24.55±0.21
30	**87.33±0.39**	**99.7±0.15**	**88.87±0.54**	**55.83±0.33**	35.02±0.35
45	95.81±0.59	**99.9±0.07**	95.38±0.3	61.32±0.43	41.2±0.78

Figure 1: Calibration plot for the estimation of Ritonavir in 0.1 N HCl

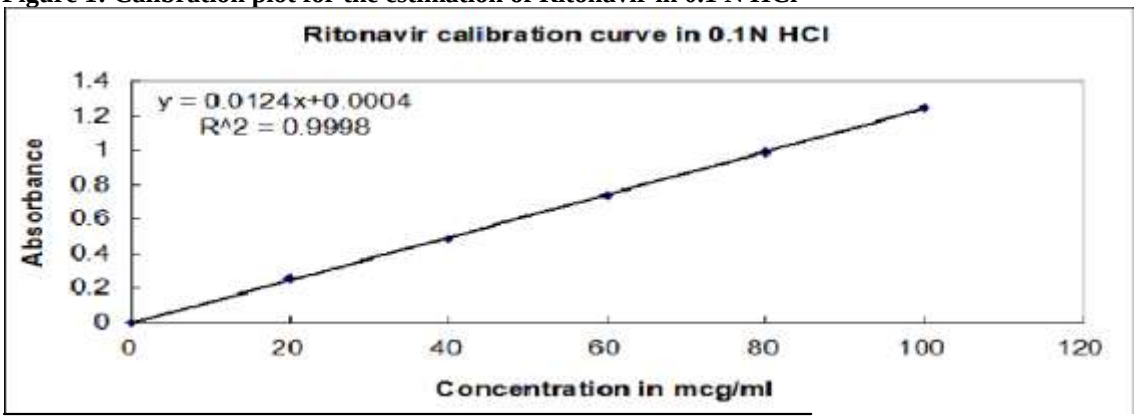


Figure 2: FTIR spectrum of Ritonavir

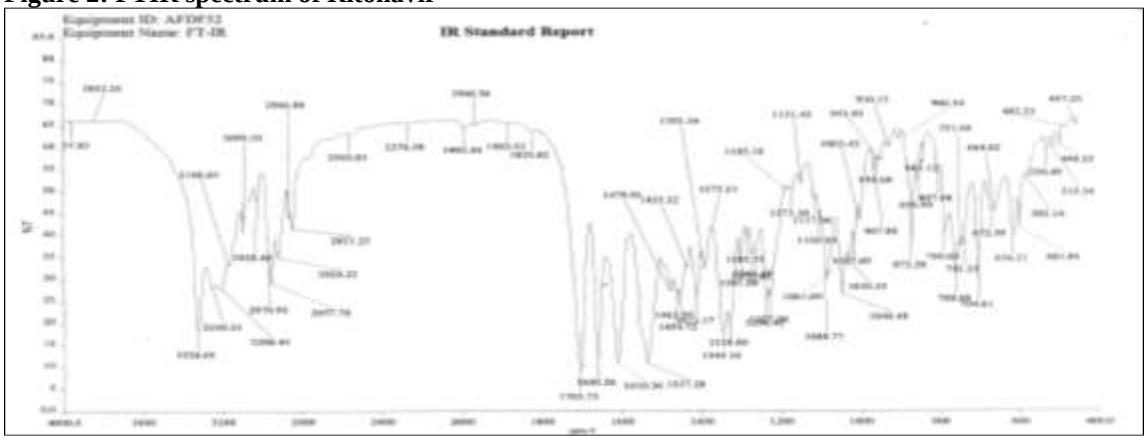


Figure 3: Pseudo ternary PD of Ritonavir with O= Oleic acid , S= Tween 80 , C= Capmul MCM at different S mix=S/C = 1:4,1:3,1:2,1:1,2:1,3:1,4:1

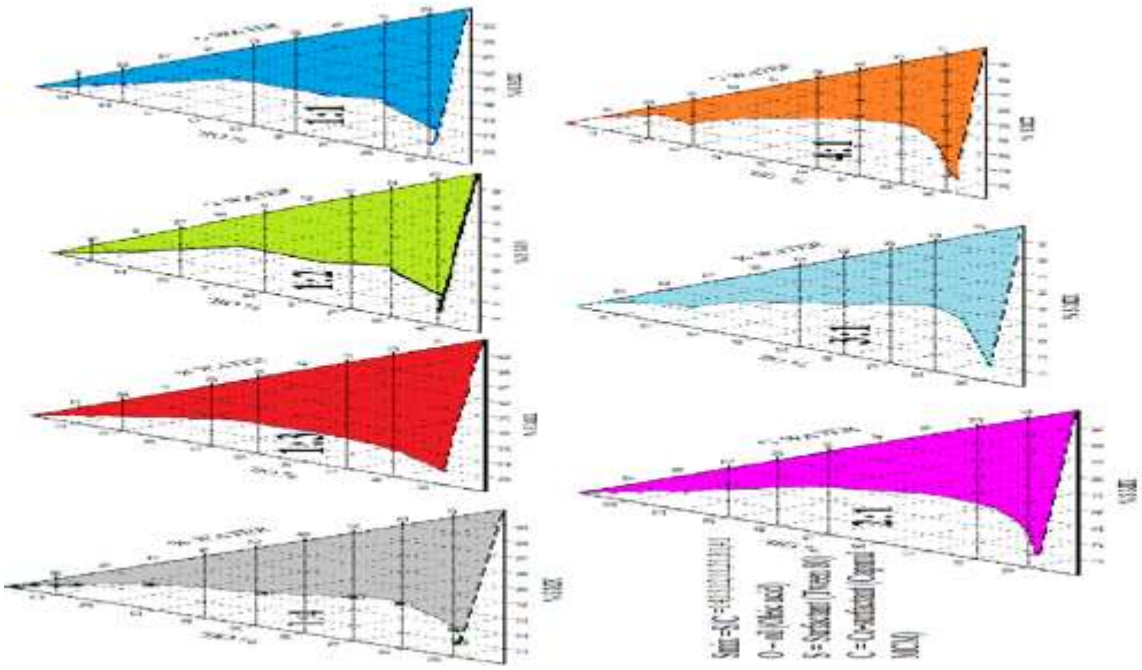


Figure 4: Formulation of Ritonavir liquid SMEDDS

Figure 5: Dissolution profiles of Ritonavir from SMEDDS (S1, S2, and S3), marketed tablet and Ritonavir drug in pH 7.0 buffer with SLS

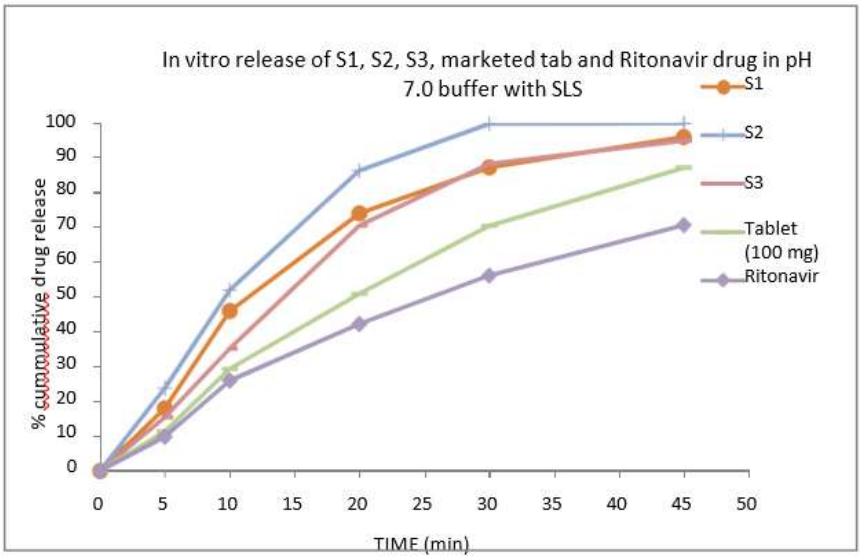


Figure 6: Dissolution profiles of Ritonavir from SMEDDS (S1, S2 & S3), marketed tablet and Ritonavir drug in pH 7.0 buffer without SLS medium

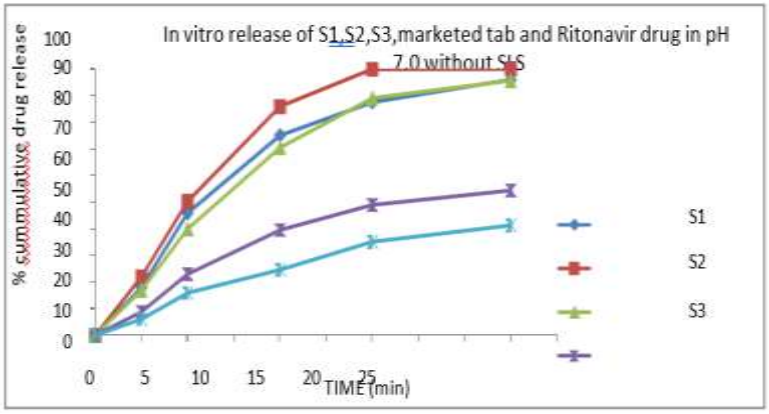


Figure 7: Dissolution profiles of Ritonavir from SMEDDS (S1, S2 & S3), marketed tablet in pH 1.2 & 7.0 buffers

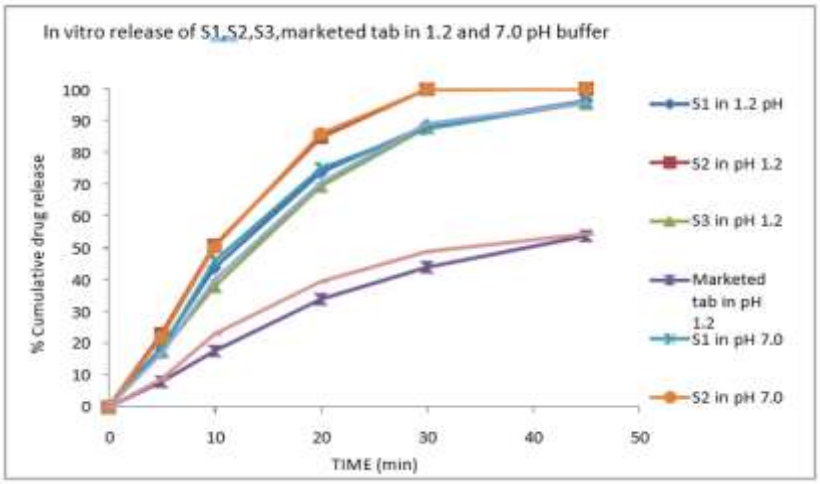
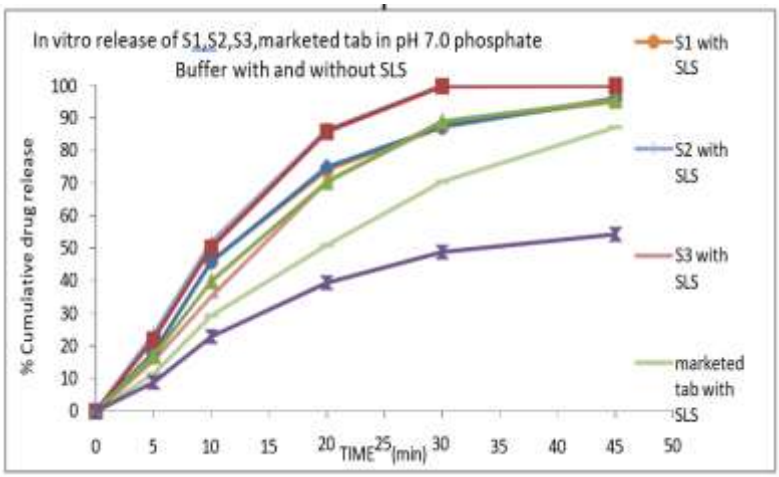


Figure 8: Dissolution profiles of Simvastatin from SMEDDS S1, S2, S3, marketed tablet in pH 7.0 buffer with & without SLS



Refractive index measurement

Refractive index of water is 1.333. All formulations were showing refractive index near to that of water at room temperature. This result together with % transmittance meaned that formulations were transparent, and no considerable difference between RI values of SMEDDS without drug and with drug, SMEDDS were thermodynamically & chemically stable.

Viscosity determination

This SMEDDS is usually administered in SGC/HGC. So, it must not be too thick but should be pourable.

Viscosity of all SMEDDS formulations without dilution were found to be in range of 212 cps to 225 cps, means that formulation possess Newtonian type flow so there is no problem infilling of formulations in hard gelatin capsule and even that is without risk of leak. As SMEDDS formulations were diluted 10 times with water, micro emulsion viscosity was decreased, it means that on oral administration of SMEDDS, it is diluted with stomach fluid and so viscosity decreases and facilitates absorption from the stomach.

In-vitro dissolution study

Drug release studies confirm the faster release of drug from formulation in dissolution medium and it also confirms self-emulsification of same formulation in medium.

In-vitro dissolution study of Ritonavir liquid SMEDDS

From the table and figures of in vitro dissolution of Ritonavir from formulations S1, S2, S3, marketed formulation and pure drug in 1.2 pH, it was found that rate and extent of Ritonavir release from all formulations, the SMEDDS formulation (S2) was considerably high compared to S1, S3, marketed tablet and pure Ritonavir drug. Maximum drug release was 99.92% in 45 min in S2 formulation in pH 1.2. From the table and figure of in vitro dissolution of Ritonavir from formulations S1, S2, S3, marketed formulation and pure drug in pH 7.0 phosphate buffer with 0.5% SLS medium, it was found that rate and extent of Ritonavir release from all formulations, S2 found considerably high compared to S1, S3, marketed tablet and pure Ritonavir drug. Maximum release of drug was 99.92% in 45 min in S2 formulation in pH 7.0 phosphate buffer with 0.5% SLS medium.

From the table and figures of in vitro dissolution of Ritonavir from formulations S1, S2, S3, marketed formulation and pure drug in pH 7.0 phosphate buffer without SLS medium, it was found that rate and extent of Ritonavir release from all formulations, S2 was considerably high compared to S1, S3, marketed tablet and pure Ritonavir drug. Maximum drug release was 99.9% in 45 min in S2 formulation in pH 7.0 phosphate buffer without SLS medium.

Here, from in vitro release data of Ritonavir in pH 1.2, pH 7.0 buffer with & without SLS, it was found that release of Ritonavir from all formulations did not affected by pH of medium. Ritonavir release from marketed tablet and pure drug was significantly affected by presence of SLS in medium, while Ritonavir release from SMEDDS S1, S2 and S3 was not affected by presence of SLS in medium. Formulation S2 showed better in vitro release in all medium.

CONCLUSION

The main study objectives are achieved that formulation and evaluation of self-emulsifying formulation containing poorly soluble drug Ritonavir. In this study liquid SMEDDS of BCS class IV drugs having low and variable bioavailability were successfully prepared. Formulation becomes more stable, reproducible, patient compliant and easy to handle without compromising its self-emulsifying property, in-vitro dissolution. By this study, it was concluded that liquid SMEDDS proved to be

a great and promising method for improvement in low solubility and low variable oral relative bioavailability for lipophilic drugs, which can be used to improve rate and the extent of relative bioavailability.

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CONFLICT OF INTEREST

Authors declare no conflict of interest

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